Psychiatric symptoms and expression of glucocorticoid receptor gene in cocaine users: A longitudinal study

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Abstract: Background Chronic cocaine users (CU) display reduced peripheral expression of the glucocorticoid receptor gene (NR3C1), which is potentially involved in stress-related psychiatric symptoms frequently occurring in CU. However, it is unknown whether psychiatric symptoms and lower NR3C1 expression are related to each other and whether reduction of drug consumption reverse them. Method At baseline, NR3C1 mRNA expression was measured in 68 recreational CU, 30 dependent CU, and 68 stimulant-naïve controls. Additionally, the Revised Symptom Checklist (SCL-90R) and the Barratt Impulsiveness Scale (BIS) were assessed. At a one-year follow-up, the association between change in NR3C1 expression and psychiatric symptoms was examined in 48 stimulant-naïve controls, 19 CU who increased and 19 CU who decreased their consumption. At both test sessions, cocaine concentrations in hair samples were determined. Mixed-effects models were used to investigate how changes in drug use intensity affect severity of psychiatric symptoms and NR3C1 expression over time. Results At baseline, recreational and dependent CU displayed elevated impulsivity and considerable symptom burden across most of the SCL-90R subscales. Time-group interaction effects were found for several impulsivity scores, SCL-90R Global Severity Index, Paranoid Thoughts, and Depression subscales as well as for NR3C1 expression. Pairwise comparisons showed that decreasing CU specifically improved in these SCL-90R subscales, while their NR3C1 expression was adapted. Finally, changes in NR3C1 expression were negatively correlated with changes in impulsivity but not SCL-90R scores. Conclusion Our findings suggest that NR3C1 expression changes and some psychiatric symptoms are reversible upon reduction of cocaine intake, thus favouring abstinence-oriented treatment approaches.

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Psychiatric symptoms and expression of glucocorticoid receptor gene in cocaine users: a longitudinal study

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Abstract

Background: Chronic cocaine users (CU) display reduced peripheral expression of the glucocorticoid receptor gene (*NR3C1*), which is potentially involved in stress-related psychiatric symptoms frequently occurring in CU. However, it is unknown whether psychiatric symptoms and lower *NR3C1* expression are related to each other and whether reduction of drug consumption reverse them.

Method: At baseline, *NR3C1* mRNA expression was measured in 68 recreational CU, 30 dependent CU, and 68 stimulant-naïve controls. Additionally, the Revised Symptom Checklist (SCL-90R) and the Barratt Impulsiveness Scale (BIS) were assessed. At a one-year follow-up, the association between change in *NR3C1* expression and psychiatric symptoms was examined in 48 stimulant-naïve controls, 19 CU who increased and 19 CU who decreased their consumption. At both test sessions, cocaine concentrations in hair samples were determined. Mixed-effects models were used to investigate how changes in drug use intensity affect severity of psychiatric symptoms and *NR3C1* expression over time.

Results: At baseline, recreational and dependent CU displayed elevated impulsivity and considerable symptom burden across most of the SCL-90R subscales. Time-group interaction effects were found for several impulsivity scores, SCL-90R *Global Severity Index*, *Paranoid Thoughts,* and *Depression* subscales as well as for *NR3C1* expression. Pairwise comparisons showed that decreasing CU specifically improved in these SCL-90R subscales, while their *NR3C1* expression was adapted. Finally, changes in *NR3C1* expression were negatively correlated with changes in impulsivity but not SCL-90R scores.

Conclusion: Our findings suggest that *NR3C1* expression changes and some psychiatric symptoms are reversible upon reduction of cocaine intake, thus favouring abstinence-oriented treatment approaches.

Introduction

Cocaine use is frequently accompanied by psychiatric symptoms (Rounsaville et al., 1991), neuropsychological deficits (Jovanovski et al., 2005; Vonmoos et al., 2013b), neurological disorders (Treadwell and Robinson, 2007) and other medical conditions, such as infectious and cardiovascular diseases (Benowitz, 1993), which all contribute to diminished quality of life and disrupted social networks, and to increased high-risk behaviour and worsened prognosis (Degenhardt and Hall, 2012; Preller et al., 2014; Whiteford et al., 2013). Particularly, the high prevalence of psychiatric symptoms in cocaine users (CU) contributes to the maintenance of substance use by inducing a more debilitated primary condition and by promoting adverse life situations (Kessler et al., 2005). This idea is supported by cumulative cross-sectional evidence associating cocaine use with, for instance, post-traumatic stress symptoms (Tull et al., 2016; Welsh et al., 2017), mood symptoms, anxiety symptoms (Roy et al., 2015), and attention-deficit/hyperactivity disorder (ADHD) (Perez de Los Cobos et al., 2011). Retrospective analyses (Glantz et al., 2009) and a few prospective studies (Zimmermann et al., 2003) (Costello et al., 2003) suggest that psychiatric disorders such as anxiety and conduct disorder can predict onset of substance use, leading researchers to assume that substance consumption might emerge as an attempt to "self-medicate" (Kessler et al., 2012; Lazareck et al., 2012). However, on the basis of cross-sectional data, it has also been proposed that affective symptoms specifically follow the onset of cocaine use, while anxiety, antisocial personality disorder and ADHD characteristics typically exist before (Rounsaville et al., 1991).

Although the self-medication hypothesis has been supported by a few prospective studies, reciprocal associations, in which cocaine use is considered as a specific predictor of later onset of psychiatric symptoms, have scarcely been investigated. A European multicentre study reported that the intensity of cocaine use predicted worse mental health, together with physical health and social vulnerability (Haasen et al., 2005). Similarly, a 6-year prospective study investigating the longitudinal effects of HIV in a CU sample found that heavy cocaine consumption was associated with an increase in depressive symptoms and that CU reported an annual worsening of depression scores (Mukerji et al., 2017). However, these previous studies are exclusively based on self-report assessments of illicit substance use, which can markedly underestimate the true substance consumption (Magura, 2010). Additionally, most longitudinal studies have not specifically investigated the effects of cocaine consumption changes on psychiatric symptoms,

instead reporting these symptoms as secondary outcomes. Therefore, it remains unclear whether psychiatric symptoms can be induced by chronic cocaine use and also be reversed if cocaine consumption is discontinued or attenuated.

Remarkably, it has been suggested that the deleterious effect of cocaine use on mental health may be partially explained by the fact that cocaine acts as a pharmacological stressor, strongly activating the hypothalamic-pituitary-adrenal (HPA) axis, one of the main stress response systems in the human body (Kirschbaum et al., 2009). Accordingly, cocaine consumption stimulates various neurotransmitter pathways, including via the activation of dopaminergic neurons, and strongly increases the release of cortisol from the adrenal glands (Deroche-Gamonet et al., 2003). The genomic effects of cortisol are mediated via the glucocorticoid receptor (GR, encoding gene *NR3C1*), which regulates the expression of target genes and is thought to be involved in the development of addiction via its interaction with the dopaminergic system (Ambroggi et al., 2009; Deroche-Gamonet et al., 2003). It has already been shown that *NR3C1* polymorphisms together with dopamine-related genetic variations have direct effects on the functioning of the prefrontal cortex (El-Hage et al., 2013), and thus possibly on the regulation of behavioural control in psychiatric disorders (Arnsten, 2006). Recently, a lower expression of *NR3C1* mRNA has been associated with cocaine use (Schote et al., 2019) and several functional variants of *NR3C1* have been associated with higher risk for depression, reduced responses after antidepressant therapy (Spijker and van Rossum, 2012), ADHD (Fortier et al., 2013; Schote et al., 2016) and cocaine addiction (Schote et al., 2019). Complementarily, it has been suggested that the glucocorticoid receptor system constitutes one of the key mechanisms affecting reward sensitivity and influencing individual susceptibility to addiction (Goeders and Clampitt, 2002), increasing the vulnerability to relapse via behavioural disinhibition (Diaz-Marsa et al., 2008; Finy et al., 2014; Maniaci et al., 2018). Accordingly, we have previously proposed that cocaine directly downregulates *NR3C1* expression by its excessive stimulation of cortisol secretion, which likely contributes to a reduced stress responsivity, increased impulsivity, and a higher burden with stress-related psychiatric disorders such as affective symptoms (Schote et al., 2019).

In this study, we therefore aimed to examine the longitudinal association between objective changes in cocaine consumption and psychiatric symptoms measured with the Revised Symptom Checklist-90 (SCL-90R) in groups of CUs with decreased (“decreasers”) or increased

cocaine use ("increasers") after a 1-year interval. Because we have previously shown that self-reported impulsivity, cluster B personality disorder symptoms, as well as facets of social and non-social cognition can covary with changing cocaine use (Hulka et al., 2015; Vonmoos et al., 2019; Vonmoos et al., 2014) – indicating that decreased cocaine use is often associated with improvements of cognitive impairments, impulsivity, and personality disorder symptoms within one year – here we expected that reducing cocaine use would also be associated with the attenuation of psychiatric symptoms as measured with the SCL-90R. Additionally, we aimed to investigate the change in peripheral expression of the *NR3C1* mRNA in response to changes in cocaine use and the relation of these changes to variations in psychiatric symptom burden. Hence, considering that i) *NR3C1* gene expression was correlated with severity of cocaine consumption (Schote et al., 2019); and ii) lowered *NR3C1* mRNA concentrations were correlated with increased total scores on the positive symptom scale of the SCL-90R in CU (Schote et al., 2019) and diagnosis of major depressive disorder (Spindola et al., 2017); we expected *NR3C1* gene expression to covary with changes in cocaine use and the severity of self-reported psychiatric symptoms including impulsivity. Finally, we aim to overcome preceding limitations arising from self-reported drug use measures by combining drug use self-reports with objective urine tests and hair toxicology analysis, as already employed in previous analyses of this sample (Hulka et al., 2015; Vonmoos et al., 2019; Vonmoos et al., 2014).

Materials and Methods

Participants

Within the context of the Zurich Cocaine Cognition Study ($ZuCo^2St$) (Vonmoos et al., 2013a), a total sample of 166 participants (98 CU and 68 stimulant-naïve controls) was analysed at baseline (T1). CU were included if cocaine was their primary drug of choice, they currently used more than 0.5g per month and were not abstinent for more than 6 months. At baseline, exclusion criteria for all participants were any current or previous neurological disorders or head injuries and severe somatic diseases. Controls were excluded if they presented with current or previous DSM-IV Axis I psychiatric disorders (except for nicotine addiction), or regular illegal drug use (>15 occasions lifetime, except for recreational cannabis use). Additional exclusion criteria for CU were use of opioids, a polytoxic drug use pattern according to DSM-IV, and

DSM-IV Axis I adult psychiatric disorders – except for cocaine, cannabis, nicotine, and alcohol abuse/dependence; history of affective disorders (current major depression was also exclusionary); and attention deficit hyperactivity disorder (ADHD).

The follow-up measurement (T2) took place approximately one year after baseline (average number of weeks between T1 and T2 for controls = 58.2, SD = 10.1; average number of weeks between T1 and T2 for CU = 62.0, SD = 14.3). One hundred and thirty-two participants agreed to be re-tested at follow-up, but 46 participants (41 CU, 5 controls) had to be excluded from the final analyses due to hair analyses revealing illegal drug use not allowed by our exclusion criteria, or a relatively stable cocaine use pattern that did not meet criteria for either increase or decrease (see below). Ultimately, 86 participants (38 chronic CU and 48 stimulant-naïve controls) were suitable for inclusion in the final analysis (Hulka et al., 2015; Vonmoos et al., 2019; Vonmoos et al., 2014).

The test procedure was similar in baseline and follow-up (Vonmoos et al., 2014). Additionally, because gene expression can be influenced by circadian rhythm (Sukumaran et al., 2010), all blood samples were collected between 10am and 2pm. Before, test sessions (T1 and T2) participants from both groups were asked to abstain from illegal substances for at least 72h and from alcohol for 24h, and compliance with these instructions was controlled using urine screenings (for technical details see Vonmoos et al., 2013). Additionally, at both test sessions participant's self-report and drug use severity were confirmed with quantitative toxicological analysis of hair samples using liquid chromatography tandem mass spectrometry (Vonmoos et al. 2014). The Cantonal Ethics Committee of Zurich approved the study (E-14/2009). All participants provided written informed consent and received compensation for their participation.

Group assignment

At baseline, because we were interested in investigating whether the severity of cocaine use was related to the severity of psychiatric symptoms, CU were characterized as either relatively pure recreational CU (RCU, n=68) or dependent CU (DCU, n=30). Cocaine dependency was diagnosed according to the Structured Clinical Interview for DSM-IV-R Axis I Disorders (SCID-I) (Vonmoos et al., 2013a). At follow-up, CU were characterized as either *increasers* (participants who consumed substantially more cocaine within 1-year follow-up, n=19) or *decreasers* (participants who consumed substantially less cocaine within 1-year follow-

up, n=19). The assignment criteria were based on the combination of absolute and relative changes in cocaine concentration in hair samples between T1 and T2, as previously described in detail by Vonmoos et al. (2014). The absolute criterion refers to a change of at least ±0.5 ng/mg in cocaine concentration according to the accepted cut-off value for reliable detection of cocaine use (Bush, 2008; Cooper et al., 2012). The relative criterion was based on a minimum increase of 20% or a minimum decrease of 10% in the hair toxicology parameter $cocaine_{total}$ (i.e., cocaine + benzoylecgonine + norcocaine) (Hoelzle et al., 2008). Cocaine increasers showed a mean increase of +30.4 ng/mg (+297%), range +0.5 to +268.5 ng/mg (+20% to +5374%), SD 61.9 ng/mg, whereas cocaine decreasers showed a mean decrease of-10.6 ng/mg (-72%), range -116.9 to -0.6 ng/mg (-100% to -12%), SD 26.7 ng/mg (Vonmoos et al. 2014).

Clinical assessments

All participants were examined by trained psychologists using the SCID-I (APA, 1994) at baseline and follow-up. At both test sessions, current (average use during the last 6 months) and lifetime (or in the last year, for T2) self-reported drug use was assessed by the structured and standardized Interview for Psychotropic Drug Consumption (IPDC; Quednow et al., 2004). The severity of psychiatric symptoms and current psychological distress was assessed by the SCL-90R (Derogatis and Cleary, 1977). The SCL-90R includes 90 items related to psychological health and contains nine subscales (Aggressiveness, Anxiety, Depression, Obsessive-compulsive, Paranoid ideation, Phobic anxiety, Psychoticism, Somatization, and Uncertainty to social contact) and three indices of global wellness (Global Severity Index [GSI], Positive Symptoms Total [PST], and Positive Symptom Distress Index, [PSDI]). The GSI is the average of all ratings, while the PST is used to report the overall number of self-reported symptoms (>0), and the PSDI indicates the average intensity of symptoms (Derogatis and Cleary, 1977). Finally, the Barratt Impulsiveness Scale (BIS) was used to assess self-reported impulsivity by extracting the total score and the three main factors: attentional, motor, and non-planning impulsiveness (Patton et al., 1995). Baseline and longitudinal results regarding the BIS in the present sample have been published previously (Hulka et al., 2015; Vonmoos et al., 2013a), however, the longitudinal analysis was done with a set of different predictors and with another reference group (increasers instead of controls) (Hulka et al., 2015).

Gene expression analysis

Total RNA was isolated from whole blood using the RNA isolation NucleoSpin RNA blood in combination with the NucleoSpin RNA/DNA buffer according to the manufacture´s recommendations (Macherey-Nagel AG, Oersingen, Switzerland). Purity and quantity of total RNA was determined spectrophotometrically (NanoVue, GE Healthcare Life Sciences), and RNA quality given as RNA integrity number (RIN) values was measured on the automated electrophoresis system (Experion, BioRad Co., Hercules, CA, USA). Per sample, 500 ng of total RNA were reverse transcribed into cDNA using the iScript cDNA synthesis kit (BioRad Co., Hercules, CA, USA). Gene expression analysis of *NR3C1* was performed using the QuantiTect SYBR green detection method (Qiagen, Hombrechtikon, Switzerland), a quantitative real-time reverse-transcription polymerase chain reaction with *NR3C1* specific detection primers (QT00012915) and four reference genes ACTB, ALAS1, GAPDH, and PPIA for normalization (Qiagen). PCR specificity was confirmed using melting curve analyses for each triplicate. Gene expression and normalization analysis was conducted using the qBase plus software (Biogazelle, The Netherlands), which uses a special algorithm originating from the geNORM (Vandesompele et al., 2002) to integrate the most stable RG together into a normalization value, for further details see Havranek et al. (2015). Furthermore, to minimize the possibility of technical issues or batch effects, both blood samples, baseline and follow-up, were analysed at the end of the study and gene expressions were assessed on the 384 well plates using the same chemicals (bath) and in a mixed manner. Moreover, PCR efficiency was determined with the program LinRegPCR (www.hartfaalcentru.nl).

Statistical analysis

Demographic data, drug use patterns, and frequency data were analysed with analyses of variance (ANOVAs), Student's t-tests, and Pearson's $\chi 2$-test using the Statistical Package for the Social Sciences 25 (SPSS 25, IBM). Spearman rank correlations were performed to explore how cocaine hair concentration values relate to use self-report consumption pattern.

Cross-sectional analyses: ANOVAs were initially performed to investigate whether stimulant-naïve controls differ from RCU and DCU concerning SCL-90R and *NR3C1* mRNA expression. Next, to investigate the estimation on the severity of cocaine consumption accessed

with hair toxicology analysis and *NR3C1* mRNA expression in both RCU and DCU a Spearman rank correlation was performed.

Longitudinal analyses: first, a series of linear mixed models (also known as linear multilevel models, random-effects, or random-coefficient models) were performed to investigate the association between changes in cocaine use, psychiatric symptoms and *NR3C1* expression over time (at T1 and T2) (Gill, 2000). Predictors (fixed-effects) evaluated were group (i.e., increasers, decreasers and controls), time (i.e., dummy variable with levels "baseline" and "follow-up"), and time-group interaction. At follow-up, because participants were asked whether they had attended any treatment (i.e., outpatients or impatient units), a dummy variable (i.e., treatment 'yes' or 'no') was included in the model as a random-effect. A random intercept effect was also modelled for each participant allowing us to control for individual mean differences at baseline (Harrison et al., 2018). Additional *post hoc* comparisons were performed using Student's t-tests and effect sizes were calculated by Pearson's correlation coefficient (r <.10 small effect size; r <.30 medium effect size; r <.50 large effect size) (Cohen, 1992).

In a second step, the same models were performed to investigate the possible effects of age, sex, years of school education, alcohol consumption, nicotine consumption, cannabis consumption, cocaine dependency diagnosis, and seasonal variation in *NR3C1* mRNA expression. Moreover, to investigate the possibility of technical issues or batch effects when performing the *NR3C1* mRNA expression, similar linear mixed models were also performed for the reference genes expression. Finally, Spearman rank correlations were performed within the increaser group (n=19), the decreaser group (n=19), and a combined user group (n=38) to investigate whether changes in cocaine consumption and changes in *NR3C1* mRNA expression were associated with changes in psychiatric symptoms.

The linear mixed models and Spearman rank correlations were performed with the open source statistical software R (R Core Team, 2015).

Results

Demographic Characteristics and Drug Use Patterns at Baseline

As previously shown for this sample (Vonmoos et al., 2013a; 2013b), controls, RCU and DCU did not differ concerning age, sex, and estimated verbal IQ (**Table S1**). Regarding the

longitudinal sample, cocaine increasers and decreasers did not significantly differ regarding age, sex, verbal IQ, years of education, smoking status, and length of interval between the two study assessments (**Table S2**). As also reported by Vonmoos et al. (2014), at baseline, increasers and decreasers displayed similar cocaine hair concentrations. However, hair analyses for increasers showed a 3-fold increase between baseline and follow-up, while decreasers displayed a quarter of the $cocaine_{total}$ hair concentration after one year (**Figure S1**). As shown in Vonmoos et al. (2013) before, Spearman rank correlations revealed that total hair concentration of cocaine metabolites ($Cocaine_{total}$) positively correlated with self-reported estimated cumulative dose (r=.518, p<.001, n=96), and duration of use (r=.386, p<.001, n=96).

Psychiatric Symptoms Severity and NR3C1 mRNA Expression at Baseline

At baseline, we found significant group differences regarding all SCL-90R subscales (**Table 1**). *Post hoc* comparisons revealed that RCU differed from controls in the main index scores GSI (**Figure 1**), PSDI and PST, as well as in the subscales of Aggressiveness, Anxiety, Compulsiveness, and Somatization, while DCU differed from controls in all symptoms except for Paranoid Thoughts and Psychoticism. Additionally, DCU differed from RCU concerning the GSI (**Figure 1**), PSDI, PST, Anxiety, Compulsiveness, Depression, somatization and uncertainty to social contact. As published before (Vonmoos et al 2013b), both RCU and DCU showed elevated BIS scores, however, values are shown again here for the sake of thoroughness and clarity.

Regarding the *NR3C1* mRNA expression, both RCU and DCU displayed lower levels than controls (**Figure 2**). This difference was only significant for RCU (26% lower than controls, r = 0.27, 95%IC = -0.94 to -0.22), although the effect size was comparable for DCU (24% lower than controls, d = 0.25, 95%IC = -1.00 to -0.05). However, when comparing the control group (n=60, mean=1.61, SD=0.76) with a combined cocaine user group (RCU and DCU) (n=88, mean=1.20, SD=0.64), we found a significantly lower level of *NR3C1* mRNA expression in the user group (t [111.55] = 3.41, p<0.000, d=-0.57, 95%IC = -0.91 to -0.24), as published previously (Schote et al. 2019). In addition to that, similarly as already shown by Schote et al. (2019), Spearman rank correlations revealed that $Cocaine_{total}$ was negatively correlated with *NR3C1* mRNA expression in the DCU group (r=-.426, p<.05, n=30), but not in the RCU (r=.043,

p=.739, n=68). This finding was expected because, when compared to RCU, DCU showed larger variability in the estimated amount of cocaine consumed (**Table S1**).

Table 1

Figure 1

Figure 2

Psychiatric Symptoms Severity and NR3C1 mRNA Expression over Time.

The longitudinal linear mixed effect models for the SCL-90R scores and *NR3C1* mRNA expression are shown in **Table 2** (for mean and SD, please see **Table S4**). Regarding symptoms, we found significant time-group interaction effects, indicating that participants who decreased their consumption over time reported less severe SCL-90R scores regarding GSI (**Figure 3a)** (p=0.026), Depression (**Figure 3b)** (p=0.015), and Paranoid thoughts (**Figure 3c)** (p=0.027), suggesting that only this group changed over time when compared to increasers and controls. Concerning between group differences at baseline and follow-up, we found that, when compared to controls, CU who decreased their consumption reported higher scores at baseline for GSI (**Figure 3a**), Depression (**Figure 3b)** and Paranoid thoughts (**Figure 3c)**, but no significant differences were found between both groups at follow-up.

Although we did not find any other significant interaction effects, the data revealed a similar pattern for all symptoms, suggesting that CU who decreased their consumption tended to improve their symptom scores, while CU who increased their consumption within one year displayed worse or stable scores on the SCL-90R subscales (**Figure S2** shows the PSI and the PDSI exemplarily). Interestingly, a group effect for CU who decreased their consumption was also found for GSI, PST, PSDI, Compulsiveness, Depression, Paranoid Thoughts, and Psychoticism scores, suggesting that they had more severe symptoms when compared to controls independently of the factor time. Similarly, we found a group effect for Aggressiveness, Compulsiveness, and Psychoticism scores for those participants who increased their consumption compared to controls. As published before (Hulka et al., 2015), we found

significant time-group interaction effect for several impulsivity scores although we used a slightly different set of predictors and firstly included treatment and ID as random effects.

Spearman correlations (Table S3) revealed that changes in cocaine hair concentrations ($Cocaine_{total}$) were negatively correlated with changes in the SCL-90R GSI, anxiety, phobia and uncertainty to social contact symptoms scores within the cocaine decrease group, suggesting that cocaine users who reduced their consumption showed more psychiatric symptoms at the follow-up. However, this correlation was driven by a single outlier with a very strong hair cocaine change score (-116.9 ng/mg) and a moderate increase in the SCL-90R scores (GSI change score +0.25). After exclusion of this participant no significant correlations between change in hair cocaine concentrations and SCL-90R scores remained in the decreasers group. Furthermore, SCL-90R somatization was positively associated with changes in cocaine hair concentrations within the cocaine increase group. Within all CU, changes in cocaine hair concentration were positively correlated with changes in the BIS total, attention impulsiveness, and motor impulsiveness scores as shown before (Hulka et al., 2015), indicating that decreased cocaine consumption went along with improvement of impulsive behaviour. Finally, again in all CU, the change in *NR3C1* mRNA expression was negatively correlated with the total score of the BIS reflecting that increased glucocorticoid receptor gene expression was associated with reduced impulsivity (**Table S3**). As shown in **Figure S4**, no correlation was found between changes in cocaine hair concentration ($Cocaine_{total}$) and NR3C1 mRNA expression within all CU (r=-.125, p=.495, n=32), DCU (r=.042, p=.907, n=10), RCU (r=-.256, p=.248, n=22), increasers (r=-.205, p=.427, n=17), nor decreasers (r=-.046, p=.869, n=15).

Table 2

Figure 3

In terms of *NR3C1* mRNA expression a time-group interaction for those participants that decreased their consumption over time was found (**Figure 4**) (p=0.049), suggesting that participants who decreased their consumption over time were approaching similar levels of *NR3C1* mRNA expression to controls at the follow-up (**Table 2**). We also found a negative group effect for both CU groups (**Table 2**), suggesting that CU have a downregulation of the

glucocorticoid receptor, as shown previously by Schote et al. (2019) for this sample. Furthermore, the negative time effect suggests that, when compared with baseline, all groups, including controls, displayed lower levels of *NR3C1* mRNA expression (**Table 2**).

Although the groups did not differ regarding age and sex, we performed the same analysis including both variables and years of education as covariates to control their effects on NR3C1 mRNA expression. Our findings revealed no effect for age, sex nor years of school education, but the time-group interaction for those participants that decreased their consumption over time was still observed (ß=.43, t[72] = 2.01, p<0.048). Additionally, because it has been recently shown that chronic alcohol consumption was associated with increased DNA methylation of the NR3C1 and reduced NR3C1 mRNA and protein expression levels in PFC (Gatta et al., 2019) and our groups differed concerning the current estimated amount of alcohol consumed during the week (in grams) (**Table S2**), we performed the same analysis including "alcohol grams per week" as a covariate. The model revealed no effect for "alcohol grams per week" (ß=.03, t[72] = 0.95, p<0.344), and the time-group interaction for those participants that decreased their consumption over time was still observed (ß=.44, t[72] = 2.06, p<0.042). Similarly, when investigating the effect of nicotine consumption in *NR3C1* mRNA expression over time, no effect was found for years of consumption (ß=.02, t[72] = 0.64, p<0.521) and amount of cigarettes per day (ß=.03, t[72] = 1.093, p<0.277). Finally, when including years of cannabis consumption in the main model, we found no effect for cannabis (ß=.02, t[72] = -0.00, p<0.999) and the time-group interaction effect remained (ß=.43, t[72] = 1.99, p=0.050). Finally, because CU increasers and CU decreasers differed regarding the number of participants diagnosed with current cocaine dependency (**Table S2**), the main model was also performed including cocaine dependency (yes/no) as a covariate. No effect was found for cocaine dependency (ß=.03, t[72] = 0.21, p<0.832) and the interaction effect of time-group remained (ß=.43, t[72] = 2.00, p<0.048) (**Figure S3** shows a linear mixed effect model performed including controls, RCU and DCU as a predictor, no time-group interaction effect was found).

Figure 4

Discussion

In this study, changes in cocaine consumption over a period of one year were linked with changes in psychiatric symptoms and *NR3C1* receptor gene expression. At baseline we found

that severity of cocaine use was related to severity of psychiatric symptoms, including impulsivity, and lower expression of the *NR3C1* glucocorticoid receptor gene. Accordingly, DCU reported higher levels of psychiatric symptoms than controls, while RCU were intermediate. CU, in general, showed lower levels of *NR3C1*, as found previously (Schote et al., 2019). However, *post hoc* analysis revealed a significant effect only for RCU on *NR3C1* gene expression. This is, nonetheless, well-explained by a reduction in statistical power, as the number of participants in the DCU group was considerably smaller (RCU, n=68; DCU, n=30) and DCU (r = 0.25) did display a similar effect size level to RCU (r =0.27). These similar effect sizes in *NR3C1* expression are surprising only at a first glance, as there was considerable overlap in the current cocaine use severity between RCU and DCU, thus confirming that the dependency criterion is not directly related to the amount of substance consumed (APA., 2013). The large within-group variability observed in the DCU may also explain why we found a negative correlation between *NR3C1* expression and cocaine hair concentrations only in this group. Together, our *NR3C1* findings are in line with our previous studies reporting that higher cocaine levels measured in hair correlate with lower *NR3C1* mRNA expression, suggesting an adaptive mechanism of cocaine dose-dependent transcriptional regulation. Therefore, our findings may support the idea that the cocaine-induced increase in cortisol levels (Baumann et al., 1995), which might lead to a sensitization of the receptor and, via feedback mechanisms, to a downregulation of *NR3C1* expression.

At follow-up, we first showed that cocaine users who decreased their cocaine consumption over time reported fewer psychiatric symptoms when compared to controls and CU who increased their cocaine use over time. These findings are independent of any treatment attendance. In the context of other substance use disorders, our results can be compared with another study in 26 adolescent cannabis users showing that a 28-day cannabis abstinence was associated with strong reductions in depressive symptoms at follow-up (Jacobus et al., 2017). Previously, Haasen et al. (2005) have shown that the intensity of cocaine use predicts worse mental health; however, the authors did not investigate whether the attenuation of use might predict better mental health. Interestingly, we found that CU who decreased consumption generally reported more severe symptoms at baseline. This finding suggests that a higher burden of psychiatric symptoms is likely a motivation to reduce consumption, given that the participants who reported fewer symptoms tended to maintain their consumption pattern or increase it.

Concerning *NR3C1* expression, our main longitudinal findings revealed that CU who decreased their cocaine consumption over time did not decrease in their *NR3C1* expression at follow-up as much as controls and CU who increased their cocaine use over time. The significant difference between decreasers and controls at baseline (effect size r=0.48) disappeared at follow-up (r=.24), while this difference remained stable and significant between increasers and controls at both time points (r=0.34). Further analyses suggest that the time-group interaction effect was not driven by nicotine, alcohol, or cannabis consumption; neither by cocaine dependency diagnosis nor seasonal factor. Additionally, the time-group interaction effect seemed to be exclusive to the *NR3C1* gene expression, since no time-group interaction effect was found for any of the reference genes. In general terms, these findings also seem to be in accordance with our interpretation of the previously demonstrated dose-response relationship between severity of cocaine use and *NR3C1* expression as a drug-induced effect (Schote et al., 2019). As discussed in Schote et al. (2019), it seems reasonable that the chronic cortisol secretion induced by cocaine (Baumann et al., 1995) in combination with elevated psychological stress levels (as described here) result in a down-regulation of the receptor (McGowan et al., 2009; Spindola et al., 2017). The glucocorticoid receptor plays a crucial role in the feedback regulation of the HPA axis (Deroche-Gamonet et al., 2003), while chronic exposure to stress (e.g., induced by psychiatric symptoms) can also lead to adaptations in this system. This may explain the lack of a significant correlation between changes in *NR3C1* expression and changes in cocaine hair concentration, given that we found that CU who decreased their consumption over time had already reported higher psychiatric symptoms and had lower levels of *NR3C1* expression at baseline when compared with the other groups.

Nevertheless, the fact that no correlation was found concerning changes in *NR3C1* expression and changes in cocaine hair concentration may also be explained by the reduced number of participants at follow-up (decreasers, n=16 and increasers, n=18) and a strong effect of time found in the linear mixed model (r=.615, p=.000). While the convergence of NR3C1 expression levels between controls and CU who decreased their consumption might suggest a normalization of *NR3C1* gene expression in cocaine decreasers, the precise effect of cocaine on *NR3C1* levels requires further investigation. Surprisingly, we observed that controls also displayed much lower levels of *NR3C1* expression at follow-up. However, this observation may be explained by a general habituation effect to the novel environment of a psychological test

setting. In other words, when participants arrived in the hospital to perform the 1-year follow-up, the environment, research team, and the test procedures were already familiar to them and, thus, perceived as less stressful overall. Additionally, the normalization procedure on the reference genes using the qBase plus software does correct for any possible changes induced by total RNA amount (e.g., cell count), reinforcing that the observed changes in NR3C1 gene expression reflect actual changes in the level of expression between baseline and follow-up induced by e.g., general habituation effects, changes in cocaine consumption, or so far unknown factors. Future studies might consider experimental stress induction challenges to elucidate the effect of changes in cocaine consumption on the HPA stress-response system and glucocorticoid receptor expression.

In line with the above interpretation, we also found that both increased *NR3C1* gene expression and decreased cocaine consumption during the study interval were associated with improvements in self-reported impulsivity across all CU. Taking together the high vulnerability for relapse that has been reported in CU – which can be understood as a lack of behavioural inhibition – and both the acute and chronic effects of regular cocaine intake (Fox et al., 2009; Grassi-Oliveira et al., 2012; McReynolds et al., 2014), these correlations may reinforce the role of glucocorticoid receptor expression in the vulnerability to relapse via increased impulsivity (Maniaci et al., 2018). Hence, our findings are in line with preceding analyses of this sample showing that cognitive impairments and elevated impulsivity were partially cocaine-induced and potentially reversible (Hulka et al., 2015; Vonmoos et al., 2014). However, the correlational nature of these findings does not allow us to provide further interpretation on the causal relationship between changes in *NR3C1* expression or cocaine consumption and self-reported impulsivity.

Our results might be interpreted in light of some limitations. Although we were able to assess a relatively large number of cocaine users twice with a 1-year interval, our sample size remained moderate for longitudinal analysis with a three-group design, which could thus possibly be underpowered. Moreover, given the small number of participants who attended any kind of treatment between baseline and follow-up (approximately 40% in each CU group), we were unable to make a clear distinction between types and intensity of treatments such as ambulatory or inpatient units. However, it must be highlighted that it was not the main goal of the present study to investigate the effect of a specific treatment on psychiatric symptoms.

Additionally, glucocorticoid receptor gene expression was measured in blood, which may not exactly reflect expression at the neuronal level. Nevertheless, correlated expression in blood and brain have been reported (Daskalakis et al., 2014), allowing at least partial interpretative transfer from the periphery to central brain functions.

Our longitudinal study investigated, for the first time, the link between changes in cocaine use over a 1-year period, psychiatric symptoms, and *NR3C1* expression levels. Based on hair toxicology analyses, we were able to ensure the inclusion of participants with little or no polysubstance use and to quantify changes in drug use over time Our main findings are: (a) CU reported higher levels of psychiatric symptoms and psychological distress compared to controls, while this effect was strongly pronounced in DCU; (b) objective changes in cocaine consumption were related to changes in psychiatric symptoms, specifically in depressive and paranoid symptoms; (c) changes in cocaine use severity are potentially associated with changes in glucocorticoid receptor expression, supporting the hypothesis that NR3C1 expression can, at least partially, be impacted by chronic cocaine exposure; and (d) longitudinal changes in glucocorticoid receptor expression were negatively correlated with changes in self-reported impulsivity, indicating that at least some cocaine-related psychiatric symptoms are explainable by substance-induced adaptions of the HPA axis.

Taken together, our findings suggest that both cocaine-related adaptations of the HPA axis and increased psychological distress can be partially reversible after discontinuation of drug use. This information can be given to users at the beginning of treatment as an incentive to reduce cocaine consumption. Nevertheless, our conclusions should be further investigated in future longitudinal studies with larger samples sizes.

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Contributors

All authors participated in the research and/or article preparation and all authors approved the final article.

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Table 1.
Psychiatric symptoms and mRNA expression at baseline.

	Controls (n=68)	RCU (n=68)	DCU (n=30)	F	df, df_{err}	*p*	$_p\eta^2$
BIS subscales							
Total Score [a]	63.40 (9.35)	68.68 (10.24) **	73.30 (12.66) ***	10.42	2, 165	.000	.113
Attention Impulsiveness [a]	14.66 (3.07)	16.35 (3.91) *	18.60 (4.13) *** +	12.55	2, 165	.000	.133
Motor Impulsiveness [a]	22.49 (3.87)	24.38 (4.46) *	25.77 (5.82) **	6.27	2, 165	.002	.071
Non-planning Impulsiveness [a]	26.25 (4.73)	27.94 (4.07)	28.93 (4.91) *	4.43	2, 165	.013	.052
SCL-90R subscales							
GSI	0.30 (0.27)	0.51 (0.44) **	0.79 (0.54) *** ++	15.85	2, 165	.000	.163
PSDI	1.18 (0.41)	1.41 (0.40) **	1.72 (0.44) *** ++	18.12	2, 165	.000	.182
PST	20.28 (0.41)	29.29 (1.41) **	38.63 (18.94) *** +	11.92	2, 165	.000	.128
Aggressiveness	0.24 (0.27)	0.59 (0.69) ***	0.74 (0.70) ***	10.72	2, 165	.000	.116
Anxiety	0.25 (0.31)	0.50 (0.52) *	0.78 (0.69) *** +	12.59	2, 165	.000	.134
Compulsiveness	0.38 (0.42)	0.64 (0.55) *	0.97 (0.68) *** +	13.30	2, 165	.000	.140
Depressive symptoms	0.44 (0.48)	0.66 (0.60)	1.00 (0.77) *** +	9.41	2, 165	.000	.104
Paranoid thoughts	0.37 (0.43)	0.59 (0.59)	0.87 (0.68)	8.72	2, 165	.000	.097
Phobia	0.11 (0.18)	0.20 (0.34)	0.37 (0.59) **	5.52	2, 165	.005	.063
Psychoticism	0.14 (0.20)	0.32 (0.38)	0.53 (0.49)	13.77	2, 165	.000	.145
Somatization	0.21 (0.20)	0.35 (0.40) *	0.60 (0.48) *** ++	12.68	2, 165	.000	.135
Uncertainty to social contact	0.39 (0.45)	0.52 (0.58)	0.87 (0.80) ** +	6.41	2, 165	.001	.079
mRNA expression							
NR3C1	1.60 (0.76)	1.19 (0.65) *	1.22 (0.60)	6.20	2, 148	.003	.079

Means and standard deviations. Significant Sidak post hoc test v. control group: *p<.05; **p<.01; ***p<.001; Significant Sidak post hoc test v. RCU group: +p<.05; ++p<.01; [a] Data already published by Vonmoos et al. (2013). Barratt Impulsiveness Scale (BIS), recreational cocaine users (RCU), dependent cocaine users (DCU), Symptom Checklist-90-R (SCL-90R), Global Severity Index (GSI), Positive Symptoms Total (PST), and Positive Symptom Distress Index (PSDI). Due to missing data, samples for NR3C1 mRNA expressions are not equal as SCL-90R subscales (RCU = 65, DCU = 27, Controls = 60).

Table 2.
Summary of the generalized linear mixed effect models.

	Fixed Effects					Random Effects
	Increaser (n=19)	Decreaser (n=19)	Time	Increase:Time	Decrease:Time	Treatment/ID
BIS subscales						
Total Score [a]	4.02 (1.46)	7.52 (2.68) **	0.10 (0.11)	2.59 (1.48)	-4.49 (-2.56) *	0.07
Attention Impulsiveness [a]	1.49 (1.57)	1.93 (1.99) *	0.27 (0.71)	0.85 (1.17)	-0.99 (-1.36)	0.06
Motor Impulsiveness [a]	0.97 (0.87)	2.91 (2.54) **	-0.60 (-1.17)	1.81 (1.85)	-2.00 (-2.04) *	0.07
Non-planning Impulsiveness [a]	1.54 (1.34)	2.66 (2.27) *	0.43 (1.12)	-0.10 (-0.13)	-1.49 (-1.99) *	0.04
SCL-90R subscales						
GSI	0.13 (1.43)	0.24 (2.57) *	-0.03 (-0.84)	0.02 (0.35)	-0.17 (-2.26) *	0.08
PST	6.54 (1.54)	11.11 (2.56) *	-2.77 (-1.53)	3.57 (1.04)	-4.39 (-1.27)	0.07
PSDI	0.12 (1.13)	0.25 (2.32) *	0.00 (0.08)	-0.00 (-0.07)	-0.20 (-1.81)	0.11
Aggressiveness	0.30 (2.16) *	0.24 (1.73)	-0.02 (-0.34)	-0.18 (-1.16)	-0.22 (-1.40)	0.11
Anxiety	0.13 (1.27)	0.17 (1.58)	-0.01 (-0.21)	0.01 (0.15)	-0.05 (-0.59)	0.10
Compulsiveness	0.25 (2.18) *	0.41 (3.47) ***	-0.04 (-0.90)	0.13 (1.32)	-0.18 (-1.87)	0.10
Depressive symptoms	0.20 (1.36)	0.38 (2.55) *	-0.03 (-0.42)	0.01 (0.13)	-0.33 (-2.48) *	0.06
Paranoid thoughts	0.09 (0.62)	0.39 (2.65) **	-0.06 (-0.93)	-0.06 (-0.53)	-0.28 (-2.24) *	0.10
Phobia	0.04 (0.62)	0.06 (0.87)	-0.05 (-1.53)	0.09 (1.27)	0.02 (0.37)	0.21
Psychoticism	0.17 (2.43) *	0.26 (3.48) ***	-0.02 (-0.72)	0.01 (0.29)	-0.10 (-1.64)	0.14
Somatization	0.08 (0.97)	0.14 (1.72)	0.00 (0.12)	0.04 (0.62)	-0.11 (-1.45)	0.08
Uncertainty to social contact	0.04 (0.35)	0.25 (1.78)	-0.10 (-1.75)	-0.04 (-0.36)	-0.17 (-1.60)	0.00
mRNA expression						
NR3C1	-0.40 (-2.68) **	-0.57 (-3.67) ***	-0.75 (-6.62) ***	0.19 (0.92)	0.43 (1.97) *	0.27
ACTB	-0.05 (-0.54)	-0.09 (-0.89)	0.01 (0.25)	0.17 (1.23)	0.24 (1.66)	0.05
ALAS1	0.05 (0.73)	-0.00 (-0.10)	-0.06 (-0.01)	0.00 (0.08)	-0.04 (-0.41)	0.02
GAPDH	0.06 (1.05)	0.08 (1.40)	-0.19 (-5.00) ***	-0.04 (-0.61)	-0.08 (-1.14)	0.40
PPIA	-0.09 (-1.16)	0.00 (0.01)	0.28 (4.69) ***	-0.13 (-1.18)	-0.10 (-0.86)	0.18

For fixed effects: coefficient (t-value) reported. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. For random effects: conditional pseudo R^2 value were estimated by subtracting the conditional pseudo R^2 value associated with the random effects of a null model from the conditional pseudo R^2 value associated with fixed effects plus the random effects of the tested model. [a] Data already published by Hulka et al. (2015) but with different predictors and reference groups. Symptom Checklist-90-R (SCL-90R), Global Severity Index (GSI), Positive Symptoms Total (PST), and Positive Symptom Distress Index (PSDI). Participant identification number (ID).

Appendix

Figure 1. Differences in SCL-90R Global Severity Index (GSI) subscale between controls and cocaine users.
Significant Sidak post hoc test: *p<.05; **p<.01; ***p<.001.

Figure 2. Differences in NR3C1 mRNA expression between controls and cocaine users.
For Student t-test, p values: * p<.05; *** p<.001.

Figure 3. Differences in psychiatric severity symptoms between controls and cocaine users for generalized linear mixed effect models.
For Linear Mixed Models: + Time-group interaction, p<.05. For Student t-test, p values: * p<.05; ** p<.01. Effect sizes were calculated by Pearson's correlation coefficient.

Figure 4. Differences in the expression of NR3C1 between controls and cocaine users.
For Linear Mixed Models: + time-group interaction, p<.05. For Student t-test, p values: * p<.05; ** p<.01. Effect sizes were calculated by Pearson's correlation coefficient.

Fig. 1

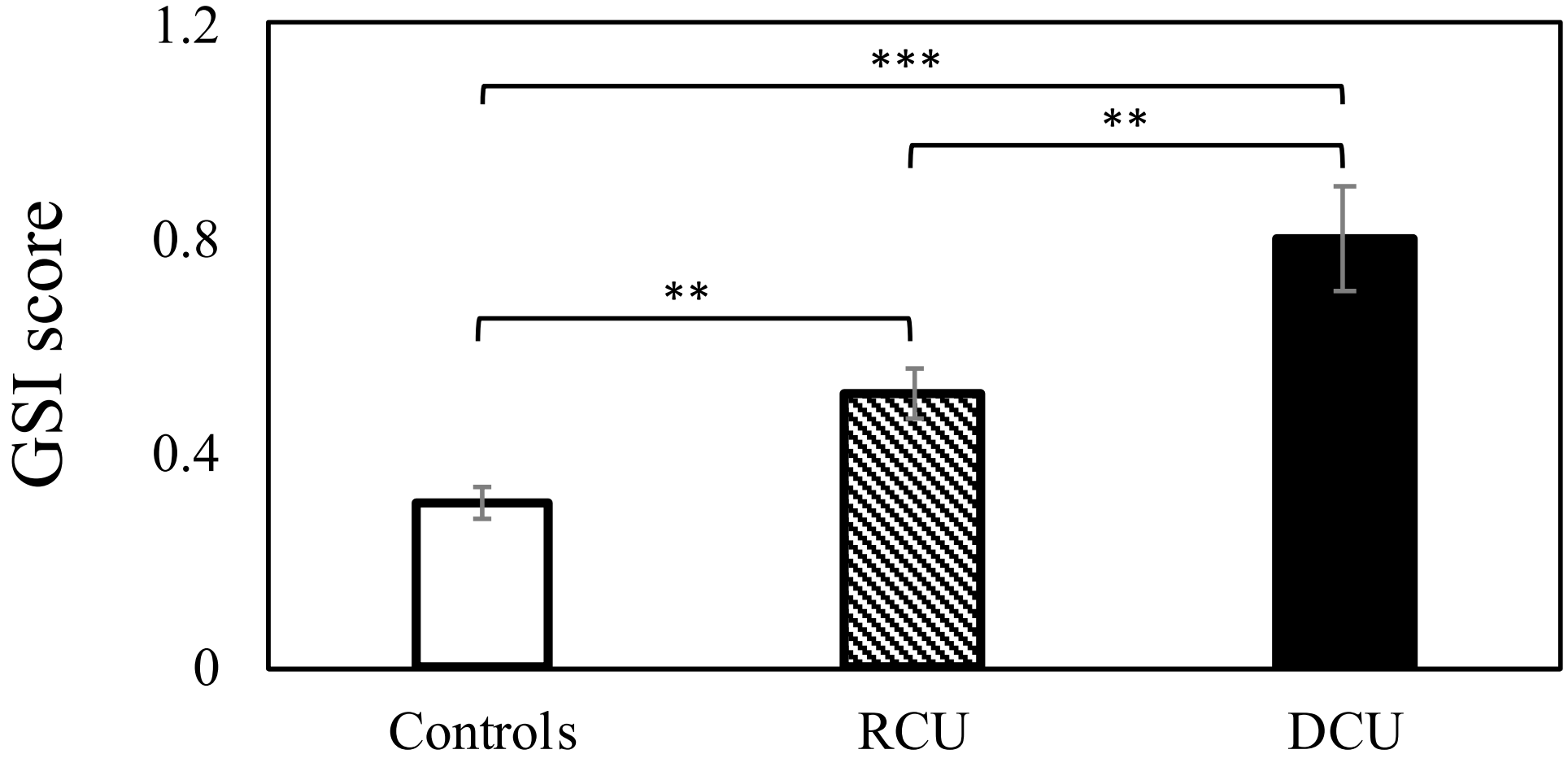


Fig. 2

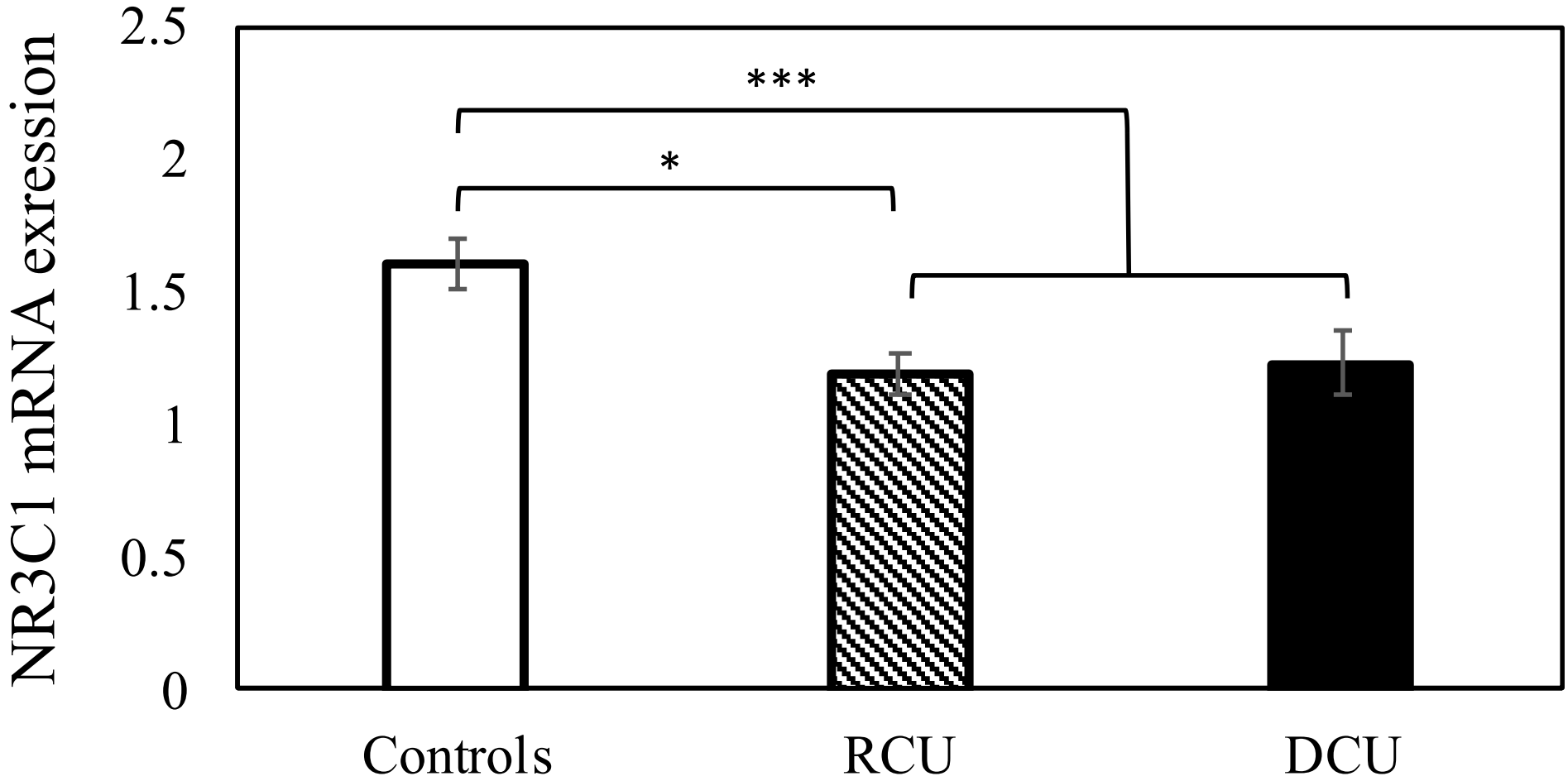
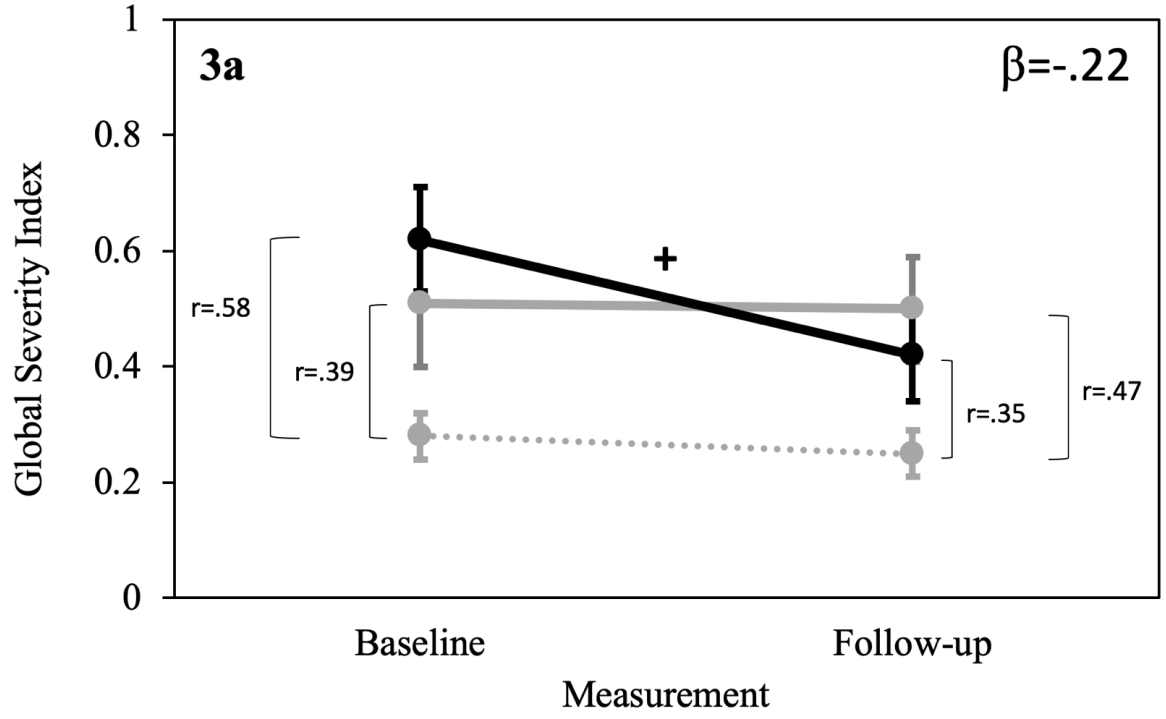
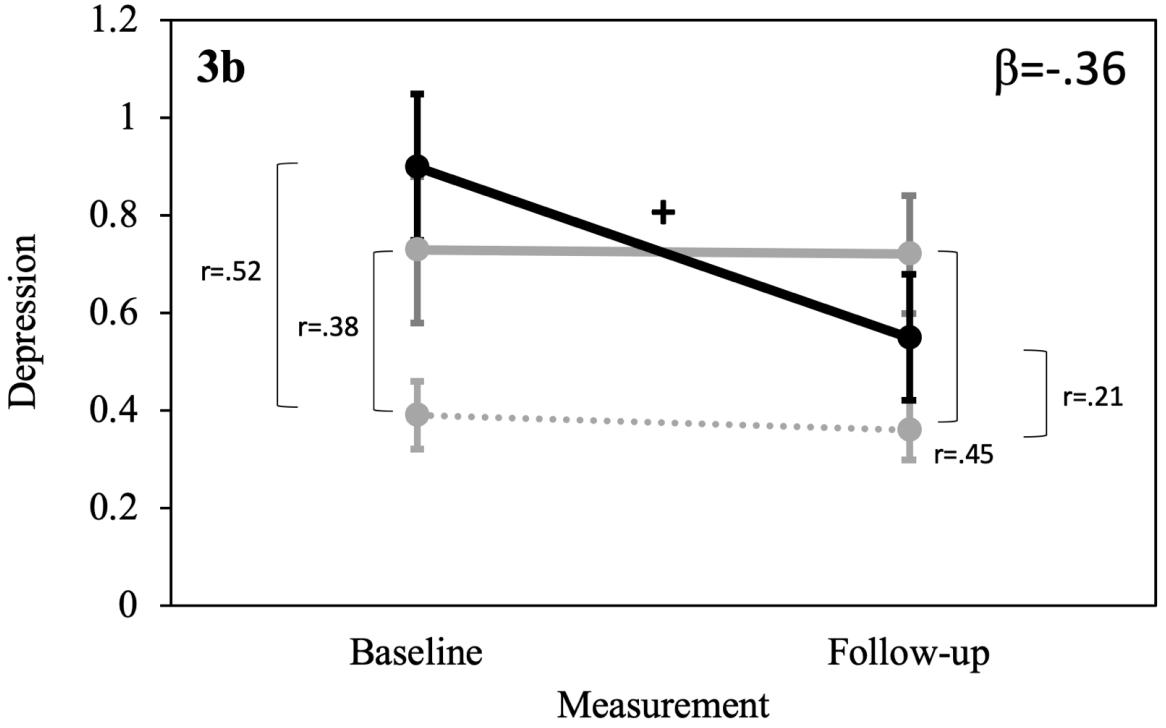
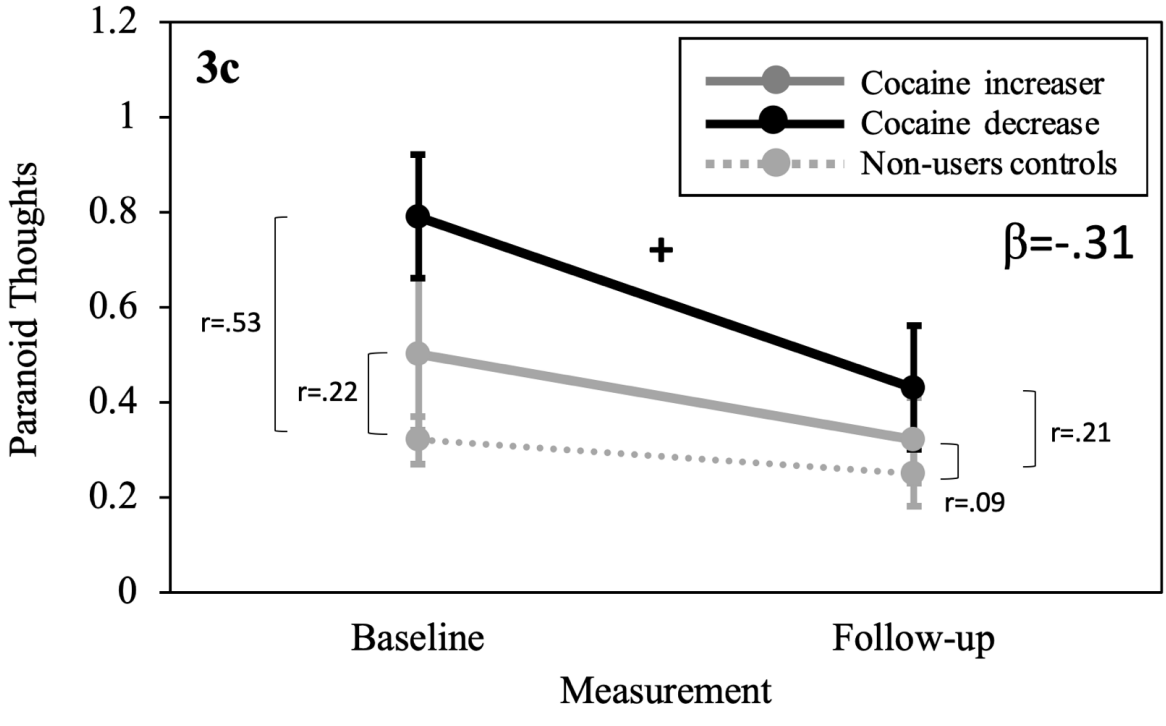


Fig. 3


3a
β=-.22
Global Severity Index
1
0.8
0.6
0.4
0.2
0
+
r=.58
r=.39
r=.35
r=.47
Baseline
Follow-up
Measurement


3b
β=-.36
Depression
1.2
1
0.8
0.6
0.4
0.2
0
+
r=.52
r=.38
r=.21
r=.45
Baseline
Follow-up
Measurement


3c
Cocaine increaser
Cocaine decrease
Non-users controls
β=-.31
Paranoid Thoughts
1.2
1
0.8
0.6
0.4
0.2
0
+
r=.53
r=.22
r=.21
r=.09
Baseline
Follow-up
Measurement

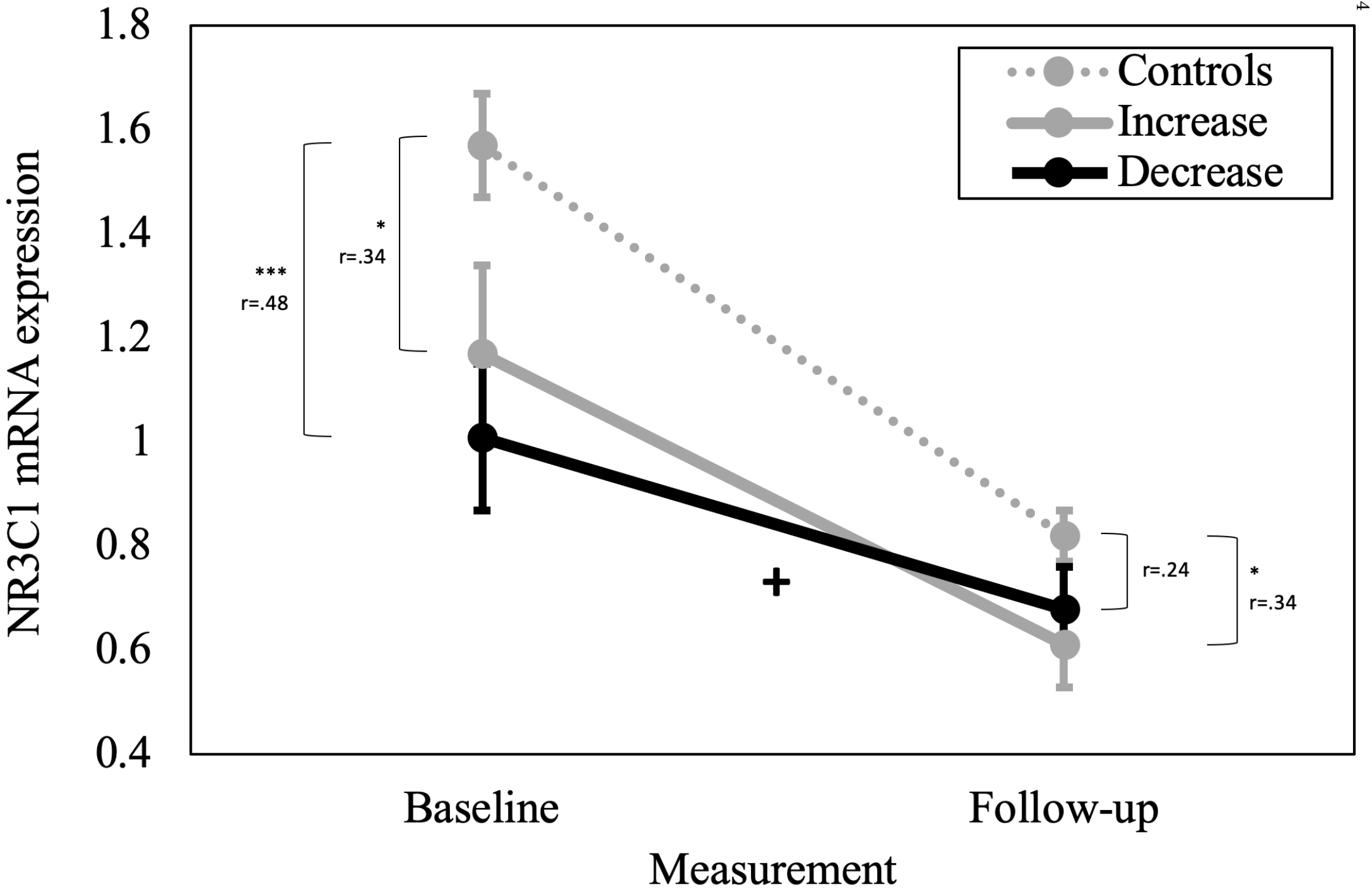

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
NR3C1 mRNA expression
Controls
Increase
Decrease
***
r=.48
*
r=.34
+
r=.24
*
r=.34
Baseline
Follow-up
Measurement

Fig. 4

Supplementary Material

Additional analysis on NR3C1 mRNA Expression over Time.

Because gene expression can be influenced by seasonal variation (Goldinger et al., 2015), we also performed the main model including season (4 levels factor variable) as a covariate. The model revealed no effect for season and the interaction effect of time-group was still observed (ß=.48, t [70] = 2.23, p<0.028). Furthermore, to investigate whether the time-group interaction was specifically observed in *NR3C1* mRNA expression, similar longitudinal linear mixed effect models were performed for the four reference genes (i.e., ACTB, ALAS1, GAPDH, and PPIA). As shown in **Table 2**, no time-group interaction for those participants that decreased their consumption over time was found. However, a positive effect for time was observed for PPIA gene expression (ß =0.28, t[73]=4.69, p<0.001), suggesting that all groups increased the expression of this gene; and an a negative effect for time was observed for GAPDH gene expression (ß =-0.19, t[73]=-5.00, p<0.001), suggesting that all groups decreased the expression of this gene.

Table S1.
Demographic data and drug consumption pattern at baseline.

	Controls (n=68)	RCU (n=68)	DCU (n=30)	Test Statistics	df, df_{err}	*p*
Age, y	30.3 (9.2)	28.7 (6.2)	32.5 (9.0)	F=2.00	2, 163	.13
Sex (f/m)	21 / 47	18 / 50	8 / 22	x^2=0.38	2	.83
Verbal IQ (MWT-B)	104.4 (9.7)	103.2 (9.6)	99.7 (9.1)	F=2.46	2, 163	.09
School education, y	10.7 (1.8)	10.5 (2.0)	9.5 (1.2) ** $^{++}$	F=4.82	2, 163	**>.05**
Craving for cocaine (0-70)	-	19.0 (9.1)	20.3 (11.4)	t=0.60	1, 96	.55
Cocaine						
Grams per week [a]	-	1.1 (1.4)	7.9 (15.8)	-	-	-
Years of use	-	6.5 (4.0)	9.4 (6.5)	-	-	-
Cumulative dose (g)	-	519.7 (751.2)	5500.9 (9635.2)	-	-	-
Last consumption (days)	-	27.5 (37.6)	21.0 (33.6)	-	-	-
Hair analysis cocaine (ng/mg)	-	2.7 (4.6)	22.2 (32.6)	-	-	-
Hair analysis benzoylecgonine (ng/mg)	-	0.6 (0.9)	5.1 (7.7)	-	-	-
Hair analysis cocaethylene (ng/mg)	-	0.3 (0.3)	2.0 (3.7)	-	-	-
Hair analysis norcocaine (ng/mg)	-	0.1 (0.1)	0.6 (0.8)	-	-	-
Hair analysis $cocaine_{total}$ (ng/mg)	-	3.4 (5.6)	27.8 (40.2)	-	-	-
Urine toxicology (n/p) [b]	68 / 0	57 / 10	18 / 12	x^2=29.07	2	**>.00**
Other substance use						
Nicotine smoking (y/n)	53 / 15	53 / 15	24 / 6	x^2=0.06	2	0.97
Nicotine cigarettes per day [a]	9.3 (9.5)	11.7 (8.7)	15.7 (13.5)	F=4.21	2	**>.05**
Nicotine years of use	9.1 (9.2)	9.6 (6.3)	14.1 (9.3)	F=4.26	2	**>.05**
Alcohol grams per week [a]	116.8 (122.6)	167.2 (116.6)	188.5 (260.6)	F=2.94	2	0.05
Alcohol years of use	13.2 (9.3)	11.2 (5.0)	13.5 (9.4)	F=1.37	2	0.25
Cannabis grams per week [a]	0.4 (0.9)	0.8 (2.0)	1.2 (3.7)	F=1.53	2	0.21
Cannabis years of use	4.6 (6.5)	7.6 (6.0)	10.5 (9.9)	F=7.85	2	**>.00**

Means and standard deviations. Significant p values are shown in bold. Sex and smoking are shown in frequency data. Significant Sidak *post hoc* test vs. control group: *p<.05; **p<.01; ***p<.001; Recreational cocaine users (RCU), dependent cocaine users (DCU). Significant Sidak *post hoc* test vs. RCU group: $^{++}$p<.01. [a] Average use during the last 6 months. [b] Urine toxicology (neg/pos) are based on cut-off value for Cocaine = 150 ng/ml and for Tetrahydrocannabinol 50 ng/ml (Substance Abuse and Mental Health Services Administration, 2008). Complete Data published on Vonmoos et al. (2013).

Table S2.
Drug consumption pattern at baseline and follow-up.

	Baseline (t1)							1-year follow-up (t2) [a]						
	Controls (n=48)	Cocaine Increaser (n=19)	Cocaine Decreaser (n=19)	F/χ^2/T	df, df_{err}	p	Effect size	Controls (n=48)	Cocaine Increaser (n=19)	Cocaine Decreaser (n=19)	F/χ^2/T	df, df_{err}	p	Effect size
Age, y	30.3 (8.9)	31.5 (9.4)	31.4 (8.3)	.20 [b]	2,83	.82	$_p\eta^2$=.00	-	-	-	-	-	-	-
Sex (f/m)	16/32	3/16	5/14	2.11[c]	2	.35	V=.16	-	-	-	-	-	-	-
Verbal IQ (MWT-B) [d]	107.6 (10.0)	102.9 (9.7)	103.8 (7.1)	2.20 [b]	2,83	.12	$_p\eta^2$=.05	-	-	-	-	-	-	-
Education, y	10.8 (1.8)	10.4 (1.8)	10.0 (1.5)	1.30 [b]	2,83	.28	$_p\eta^2$=.03	-	-	-	-	-	-	-
Weeks between t1 and t2	58.2 (10.1)	59.3 (12.1)	61.9 (14.5)	.69 [b]	2,83	.50	$_p\eta^2$=.02	-	-	-	-	-	-	-
Season at measurement time [i]	8/5/16/19	5/3/6/5	4/6/6/3	-	-	-	-	11/8/12/17	5/4/4/6	2/12/4/1	-	-	-	-
Cocaine														
Times per week [e]	-	1.6 (1.8)	1.0 (1.3)	1.17 [f]	36	.25	d=.38	-	1.1 (0.8)	0.3 (0.3)	3.85 [f]	36	**<.001**	d=1.32
Grams per week [e]	-	2.0 (2.5)	1.7 (2.3)	.41[f]	36	.68	d=.12	-	1.6 (2.5)	0.4 (0.4)	2.18 [f]	36	**.04**	d=.67
Years of use	-	7.0 (5.5)	8.2 (5.4)	.68 [f]	36	.50	d=.22	-	8.9 (5.4)	9.7 (5.2)	.45 [f]	36	.65	d=.15
Cumulative dose (grams) [e]	-	1182 (1635)	3698 (8585)	1.25 [f]	36	.22	d=.41	-	91 (119)	49 (89)	1.25 [f]	36	.22	d=.40
Last consumption (days)	-	18.5 (25.1)	16.8 (14.6)	.29 [f]	36	.77	d=.08	-	7.0 (6.3)	81.4 (145.1)	2.23 [f]	36	**.03**	d=.72
Hair analysis (ng/mg)														
$Cocaine_{total}$	-	10.3 (29.2)	14.9 (32.2)	.46 [f]	36	.65	d=.15	-	40.7 (76.1)	4.2 (8.2)	2.08 [f]	36	**.05**	d=.67
Cocaine	-	8.2 (23.3)	11.4 (23.9)	.42 [f]	36	.68	d=.14	-	31.7 (56.5)	3.1 (5.9)	2.19 [f]	36	**.03**	d=.71
Benzoylecgonine	-	1.9 (5.5)	3.1 (7.6)	.58 [f]	36	.56	d=.18	-	8.3 (19.6)	1.0 (2.2)	1.62 [f]	36	.11	d=.52
Cocaethylene	-	1.0 (2.8)	0.9 (2.8)	.11 [f]	36	.91	d=.04	-	1.2 (2.1)	0.3 (1.0)	1.56 [f]	36	.13	d=.55
$Norcocaine_t$	-	0.2 (0.5)	0.4 (0.8)	.83 [f]	36	.41	d=.30	-	0.6 (1.4)	0.1 (0.1)	1.71 [f]	36	.10	d=.50
Urine toxicology (n/p) [g]	48/0	14/5	16/3	.63 [f]	1	.43	V=.13	48/0	7/12	18/1	14.15 [c]	1	**<.001**	V=.61
Dependency (DSM-IV) (y/n)	-	-	-	-	-	-	-	-	10/9	2/17	7.79	1	<.01	V=.03
Alcohol														
Grams per week [g]	119.9 (136.8)	169.4 (129.2)	155.3 (146.4)	1.07 [b]	2,83	.35	$_p\eta^2$=.03	104.3 (88.6)	259.7 (244.5) ***	127.4 (141.4) +	7.71[b]	2,83	**<.001**	$_p\eta^2$=.16
Years of use	13.3 (8.8)	13.7 (7.6)	12.0 (7.3)	.23 [b]	2,83	.79	$_p\eta^2$=.01	14.0 (8.7)	14.8 (7.5)	12.6 (7.9)	.34 [b]	2,83	.71	$_p\eta^2$=.01
Nicotine														
Smoking (y/n) [g]	37/11	14/5	14/5	.13 [c]	2	.94	V=.04	40/8	15/4	13/6	1.83 [c]	2	.40	V=.15
Cigarettes per day [g]	8.7 (8.7)	12.8 (11.2)	9.5 (8.2)	1.38 [b]	2,83	.26	$_p\eta^2$=.03	8.2 (8.7)	13.4 (12.0)	8.2 (7.8)	2.31 [b]	2,83	.11	$_p\eta^2$=.05
Years of use	9.3 (8.3)	10.4 (8.9)	12.7 (10.3)	.95 [b]	2,83	.39	$_p\eta^2$=.02	10.5 (8.8)	12.5 (8.6)	12.6 (9.9)	.56 [b]	2,83	.57	$_p\eta^2$=.01
Cannabis														
Grams per week [g]	0.6 (1.6)	3.3 (8.9)	1.2 (2.3)	2.38 [b]	2,83	.10	$_p\eta^2$=.05	0.5 (1.6)	2.1 (4.6)	1.1 (2.7)	2.28 [b]	2,83	.11	$_p\eta^2$=.05
Years of use	4.5 (4.9)	9.5 (8.5) *	10.1 (9.7) *	5.92 [b]	2,83	**.004**	$_p\eta^2$=.12	4.6 (5.9)	10.5 (9.8) *	8.6 (9.7)	4.64 [b]	2,83	**.01**	$_p\eta^2$=.10
Cumulative dose (grams)	980 (3985)	3199 (5899)	2606 (6359)	1.61[b]	2,83	.21	$_p\eta^2$=.04	53.4 (180)	217.8 (526.5)	84.7 (189.6)	2.15 [b]	2,83	.12	$_p\eta^2$=.05

Note. Means and SD. Significant p values are shown in bold. [a]Parameters at follow-up refer to the 1-year period between t1 and t2. [b]ANOVA (all groups, with significant Sidak post hoc test vs control group: p<0.05; **p<0.01; ***p<0.001; vs cocaine increaser: +p<0.05). [c]w^2-test (all groups/cocaine users only) for frequency data. [d]Verbal IQ was assessed by the Mehrfachwahl Wortschatz Intelligenztest (Lehrl, 1999). [e]Average use during the last 6 months. [f]Independent t-test (cocaine users only). [g]Urine toxicology (neg/pos) are based on cutoff value for Cocaine 1⁄4 150 ng/ml and for Tetrahydrocannabinol 50 ng/ml (Substance Abuse and Mental Health Services Administration, 2008). The w^2-test for cocaine includes only cocaine users, the w^2-test for cannabis includes controls and cocaine users. [h]Last consumption is averaged only for persons who used the drug in the last 6 months. [w]winter/spring/summer/autumn.

Table S3.
Spearman correlations between changes in cocaine consumption, mRNA expression, symptoms and impulsiveness.

	Increaser		Decreaser		Both groups	
	$Cocaine_{total}$	NR3C1	$Cocaine_{total}$	NR3C1	$Cocaine_{total}$	NR3C1
SCL-90R subscales changes scores						
GSI			-.479 *			
PSDI	.408		-.451			
PST					.283	
Aggressiveness						
Anxiety			-.509 *			
Compulsiveness					.292	
Depressive symptoms						
Paranoid thoughts						
Phobia		.438	-.578 **			
Psychoticism						
Somatization	.575 *		-.417			
Uncertainty to social contact			-.466 *			
Impulsivity changes scores						
BIS Total Score					.414 *	-.344 *
BIS Attention Impulsiveness					.344 *	
BIS Motor Impulsiveness					.335 *	-.308
BIS Non-planning Impulsiveness						

Only correlations p<.09 are shown. *p<.05; **p<.01.

5
6

Table S4.
Means and standard deviation of the main outcome variables.

	Baseline			Follow-up		
	Control	Increaser	Decreaser	Control	Increaser	Decreaser
BIS subscales						
Total Score [a]	63.98 (8.61)	68 (14.27)	72.63 (10.10)	64.08 (8.9)	70.61 (11.83)	67.16 (9.66)
Attention Impulsiveness [a]	14.40 (3.31)	15.89 (4.57)	16.42 (3.58)	14.67 (2.76)	17.06 (4.26)	15.58 (3.66)
Motor Impulsiveness [a]	22.92 (3.61)	23.89 (5.84)	26 (2.29)	22.31 (3.70)	25 (4.31)	23.37 (4.04)
Non-planning Impulsiveness [a]	26.67 (3.83)	28.21 (5.14)		27.10 (3.92)		
SCL-90R subscales						
GSI	.28 (.25)	.51 (.48)	.62 (.39)	.25 (.25)	.50 (.39)	.42 (.34)
PSDI	1.18 (.38)	1.42 (.40)	1.55 (.42)	1.18 (.41)	1.41 (.32)	1.35 (.31)
PST	18.79 (12.46)	29.05 (19.21)	33.58 (15.67)	16.02 (12.64)	29.61 (17.34)	26.21 (15.86)
Aggressiveness	.23 (.24)	.77 (.97)	.76 (.76)	.20 (.23)	.53 (.56)	.46 (.55)
Anxiety	.23 (.27)	.45 (.49)	.48 (.40)	.22 (.32)	.46 (.42)	.43 (.44)
Compulsiveness	.33 (.37)	.59 (.56)	.75 (.55)	.29 (.30)	.67 (.50)	.52 (.46)
Depressive symptoms	.39 (.46)	.73 (.63)	.90 (.66)	.36 (.42)	.72 (.53)	.52 (.55)
Paranoid thoughts	.32 (.38)	.50 (.69)	.79 (.59)	.25 (.48)	.32 (.39)	.43 (.55)
Phobia	.14 (.20)	.18 (.30)	.19 (.32)	.08 (.22)	.21 (.34)	.17 (.25)
Psychoticism	.11 (.16)	.29 (.48)	.36 (.33)	.09 (.13)	.28 (.40)	.23 (.27)
Somatization	.22 (.21)	.40 (.43)	.46 (.35)	.22 (.20)	.46 (.47)	.37 (.26)
Uncertainty to social contact	.36 (.43)	.50 (.65)	.69 (.59)	.25 (.38)	.36 (.63)	.42 (.42)
mRNA expression						
NR3C1	1.57 (.68)	1.17 (.71)	1.01 (.59)	.82 (.36)	.61 (.35)	.68 (.32)
ACTB	1.04 (1.04)	.98 (.27)	.94 (.25)	1.06 (.42)	1.18 (.37)	1.21 (.43)
ALAS1	1.04 (.22)	1.10 (.24)	1.06 (.32)	1 (.34)	1.15 (.74)	.93 (.22)
GAPDH	1.05 (.16)	1.13 (.23)	1.2 (.49)	.88 (.22)	.90 (.25)	.85 (.13)
PPIA	1.01 (.36)	.90 (.23)	1 (.32)	1.32 (.38)	1.07 (.35)	1.19 (.30)

Note. [a] Data already published by Hulka et al. (2015);

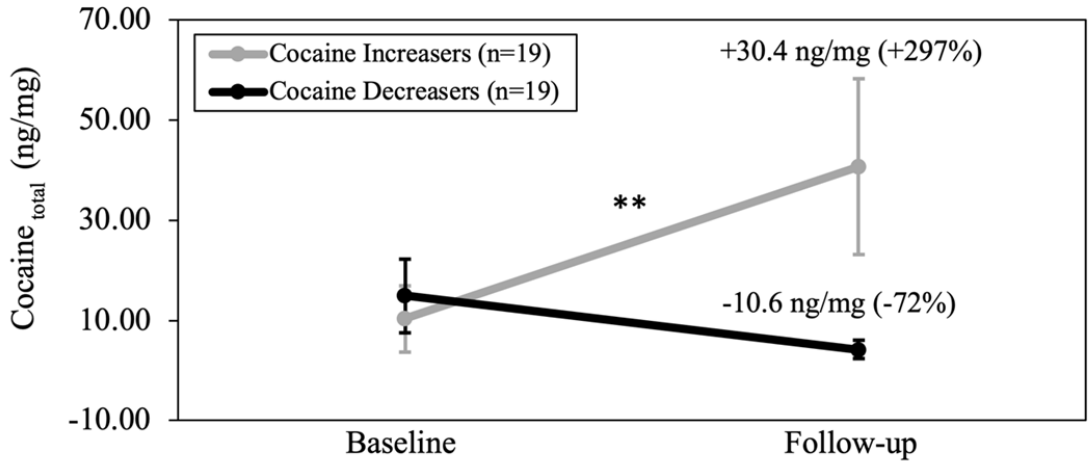


Figure S1. Hair concentration cocaine$_{total}$ in three cocaine user groups at baseline and one-year follow-up. Hair concentration cocaine$_{total}$ (ng/mg) in cocaine user groups. Means and standard deviation. A mixed design analysis (ANOVA) showed a significant time-group interaction effect ($F[2,54]=5.70$, $p<.10$). Significant Sidak *post hoc* test ** $p<.01$. Data already published on Vonmoos et al. (2014).

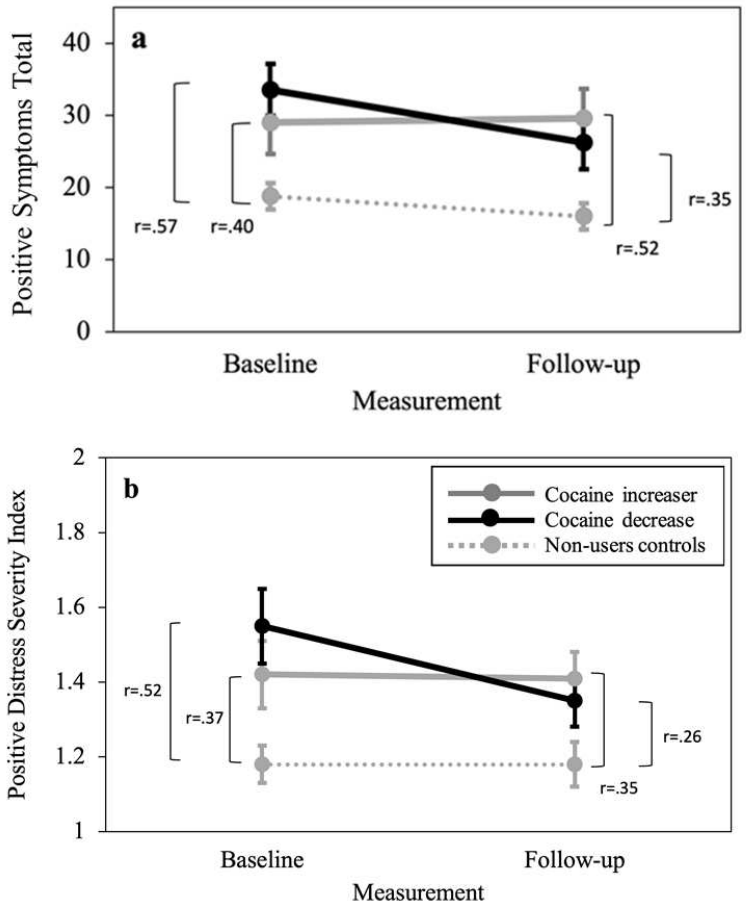


Figure S2. Differences in psychiatric severity symptoms between controls and cocaine users for generalized linear mixed effect models. Effect sizes were calculated by Pearson's correlation coefficient.

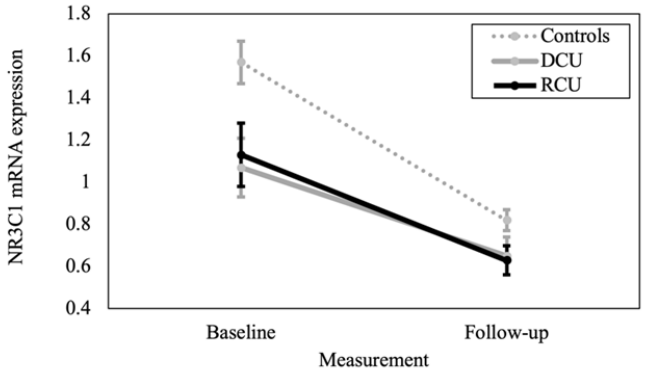


Figure S3. Differences in NR3C1 mRNA expression between controls and cocaine users. No difference between DCU and RCU was found, neither time-group effect when performing a generalized linear mixed effect model.

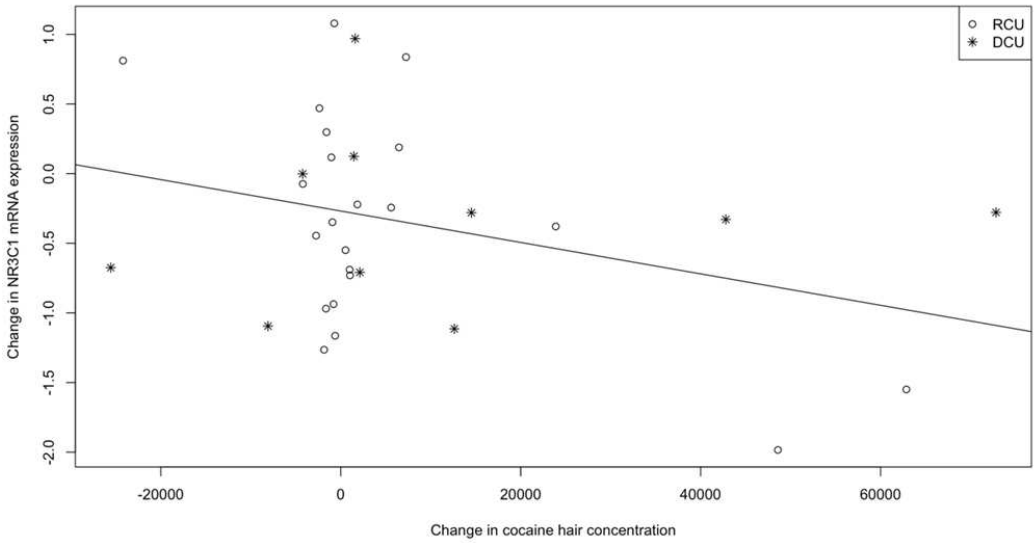


Figure S4. Scatter plot of changes in cocaine hair concentration ($Cocaine_{total}$) and NR3C1 mRNA expression. No significant correlation was found within all cocaine users (r=-.125, p=.495, n=32), DCU (r=.042, p=.907, n=10), RCU (r=-.256, p=.248, n=22), increasers (r=-.205, p=.427, n=17), nor decreasers (r=-.046, p=.869, n=15).